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TECHNICAL MANUSCRIPT 558

NOSEMATOSIS IN LABORATORY ANIMALS

William C. Hall

DECEMBER 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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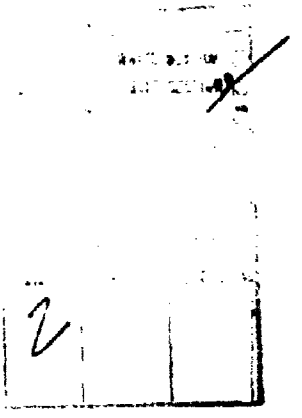

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NOSEMATOSIS IN LABORATORY ANIMALS

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Pathology Division
MEDICAL SCIENCES LABORATORIES

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December 1969

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

A review of the literature and personal observations on nosematosis of laboratory rodents are presented. The disease, caused by the protozoan Nosema cuniculi, is a spontaneous enzootic entity of laboratory rodents. The presence of occult infections in laboratory animals has confounded the results of numerous experiments. The importance of diagnosing nosematosis in laboratory animals, therefore, cannot be overemphasized. Clinical signs are usually absent, and infection can be recognized only by necropsy or by recovery of the organism following intraperitoneal inoculation of infected material into mice. Gross and histopathological lesions are described for various laboratory animals. The mode of natural transmission of the disease has not been determined. There is a need for further research on transmission and control of nosematosis as well as in vitro culture of the organism.

I. INTRODUCTION*

Nosematosis is a parasitic disease of laboratory rodents, rabbits, dogs, and man, caused by the protozoan Nosema cuniculi. The disease was first described in 1922 by Wright and Craighead,[1] who recognized a fatal paralytic disease in control rabbits used in an experiment on infantile paralysis. Lesions associated with N. cuniculi have complicated drug studies in rabbits,[2] tumor transmission studies in white rats and mice,[3] studies utilizing the rabbit in an attempt to reproduce viral diseases,[4] and perhaps other experiments where nosematosis was not recognized. Nosema has been incriminated as the cause of a disease in man[5] and is commonly recognized as the cause of a disease in both bees and silkworms.[6]

This paper describes the epidemiology, clinical signs, pathology, characteristics of the organism, and differential diagnostic considerations in laboratory animals infected with N. cuniculi.

II. DESCRIPTION

A. EPIDEMIOLOGY

Nosematosis is commonly thought to be a disease enzootic to an animal colony. Of 900 laboratory rabbits necropsied from 1940 to 1953, Robinson[7] reported that 54% of the animals present in the colony longer than 6 months were infected, in contrast to 21% of the animals present less than 6 months. He also reported a greater prevalence in female rabbits, but this has not been substantiated by other workers. Oliver[8] reported about 20% infected in an unspecified number of laboratory rabbits. There also may be a higher prevalence of the disease in albino rabbits.[7,8]

Similar results have been reported for certain colonies of rats and mice. Attwood and Sutton[10] reported that 20% of 365 adult albino Wistar rats in their colony had lesions of nosematosis at necropsy. Innes and Saunders[11] cited another worker who in a study on carcinogenesis found N. cuniculi with and without lesions in about 50% of several hundred mouse brains.

Most workers in the field believe that the disease is transmitted by ingestion of infected urine.[1,4,7,8,11] In a controlled experiment, Nelson[13] showed that parenteral injection of blood, urine, spleen, brain, kidney, liver, or lung from infected mice into noninfected mice produced

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the disease. He also demonstrated oral and intranasal transmission of the disease to weaned and suckling mice with ascitic fluid from infected mice. Congenital transmission[14] and coital spread[7] may also occur. The organism is generally considered to be pathogenic for all laboratory rodents.[4]

B. CLINICAL SIGNS

The clinical disease in laboratory rodents is usually occult. Wright and Craighead described a syndrome of paralysis and death, but most observers consider this an exception. Occasionally convulsions or tremors may occur.

C. GROSS PATHOLOGY

Gross changes are limited mainly to the kidney and aortic arch. The kidneys are frequently enlarged and the cortical surface is pitted with pale foci 1 to 3 mm in diameter. On cut section, pale streaks extend from these depressions into the medulla.

The aortic arch is often slightly dilated, and rough encrusted plaques may be found in the vessel wall. These plaques consist of mineral deposits that accompany the aortic inflammation.[7] No other gross lesions are seen.

D. HISTOPATHOLOGY

1. Brain

The most striking morphologic changes are present in the brain. According to Perrin,[14] microscopic changes can be expected as early as 8 days following inoculation with N. cuniculi. These are confined for the most part in all species to the cerebrum and pons, with lesions rarely demonstrable in the cerebellum and medulla. The histologic pattern varies with the species of animal. Rabbits show focal granulomas as large as 500 μ in diameter (Fig. 1). Epithelioid cells and a few lymphocytes are the primary cellular components of the lesions. Central necrosis and, on rare occasions, a giant cell can be found in older lesions. All granulomas lie in the vicinity of a blood vessel. Perivascular cuffing with lymphocytes about 1 to 10 cells deep occurs around vessels in proximity to the lesion. Meningeal involvement is frequently observed.

The pathologic process in mice is essentially a meningoencephalitis of vascular origin. Granuloma formation is the exception rather than the rule compared with that in rabbits and rats. Usually perivascular infiltration with lymphocytes, one to three cells thick, occurs around vessels in a focus a few hundred μ in diameter. There may be a diffuse microglial reaction that accompanies the perivascular lesions. Meningeal reactions and the distribution of lesions are otherwise similar to those in rabbits and rats.[11]

The lesion in the rat is composed mainly of lymphocytes with a moderate number of epithelioid cells scattered throughout the involved focus. Central necrosis may occur and the distribution of lesions is similar to that in the other two species described.[3]

Occasionally pseudocysts may be found in areas of little or no tissue reaction. These are as large as 70 μ in diameter and contain numerous slightly refractile organisms, which give the pseudocyst a granular appearance (Fig. 2). The individual organisms can be readily demonstrated by means of the special stains discussed later.

2. Kidney

Basophilic streaks can be seen that extend from the capsular depressions to the corticomedullary junction and frequently into the medulla (Fig. 3). Glomeruli are intact; tubules are atrophic and replaced by connective tissue and mononuclear leukocytes. In acute cases, organisms may be found in distended tubular epithelial cells, particularly in the medulla (Fig. 4 and 5). Polymorphonuclear leukocytes are usually seen in the interstitial tissue adjacent to these pseudocysts. Hyaline and granular casts are often found in tubular lumens.

3. Heart

Most animals have a focal interstitial myocarditis characterized by mononuclear accumulations about 20 to 30 μ in diameter. Myocardial fibers are sometimes separated by edema and there is degeneration of muscle cells adjacent to inflammatory foci.

4. Aorta

The aortic lesion is primarily a focal inflammation of the media that consists of a few round cells and mineral deposits (Fig. 6). It involves only the aortic arch and not the aortic valve or other parts of the thoracic or abdominal aorta. This lesion is not always seen but is most constant in older severely affected rabbits.[7]

5. Liver

When present, the hepatic lesions are a periportal hepatitis characterized by accumulations of round cells in portal areas. Occasional foci are seen in the parenchyma.

6. Other Tissues

According to some authors, pulmonary tissues have focal accumulations of lymphocytes adjacent to bronchioles. The spleen may be laden with macrophages.[4,13,14] No other lesions have been reported.

FIGURE 1. Granuloma in the Hippocampus of a Rabbit Brain. Hematoxylin and eosin, 130X.

FIGURE 2. Pseudocysts Containing Nosema cuniculi in Rabbit Brain. Hematoxylin and eosin, 420X.

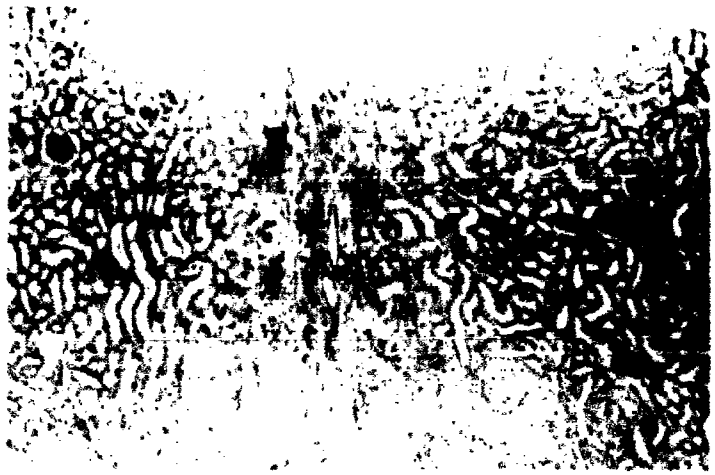

FIGURE 3. Chronic Renal Lesion in a Rabbit. Note the capsular depression and focal scarring of the cortex. Casts are present in tubular lumens. Hematoxylin and eosin. 42X.

FIGURE 4. Epithelium of Renal Collecting Ducts Distended by Large Numbers of N. cuniculi. Hematoxylin and eosin, 420X.

FIGURE 5. Epithelium of Renal Collecting Ducts Distended by Large Numbers of N. cuniculi. Brown and Brenn, 420X.

FIGURE 6. Aortic Arch of Rabbit. Inflammation and Mineralization of the Tunica Media. Hematoxylin and eosin, 330X.

E. CHARACTERISTICS OF NOSEMA CUNICULI AND DIFFERENTIAL DIAGNOSES

In tissue sections stained with hematoxylin and eosin, N. cuniculi spores are poorly stained and difficult to identify. However, mature spores are strongly gram-positive and can be identified readily with any tissue Gram stain. Perrin's modification of Goodpasture's carbolfuchsin and Brown and Brenn are excellent stains for this purpose. The spores are elongated, both ends are rounded, and they measure approximately 0.7 to 1 by 1.5 to 2.5 μ. A rigid capsule surrounds each spore. One or two polar vacuoles are often observed in the cytoplasm. The function of these vacuoles is not known; they may contain the sporoplasm or infective portion of the protozoan. This is connected to a filament, which under proper conditions ejects the sporoplasm from the spore and into another host cell where development occurs.[12]

Toxoplasma spp. have frequently been confused with N. cuniculi; this is the etiologic differentiation to be made.[15] These organisms can easily be distinguished on the basis of morphology and staining reactions (Table 1). Other organisms that may be confused with N. cuniculi can also be differentiated by morphological and histochemical characteristics (Table 2).

TABLE 1. COMPARISON BETWEEN NOSEMA CUNICULI AND TOXOPLASMA SPP. IN TISSUE SECTION

Characteristic or Stain	Nosema cuniculi	Toxoplasma spp.
Size	0.7 to 1.0 μ by 1.5 to 2.5 μ	1.5 to 2.5 μ by 2.5 to 4 μ
Shape	Rod-shaped Blunt ends	Crescent-shaped One sharp end
Hematoxylin & eosin	Organisms negative Background clear	Organisms basophilic Background eosinophilic
Gram stain	Positive	Do not stain
PAS	Weak	Strong

TABLE 2. DIFFERENTIAL DIAGNOSES IN LABORATORY ANIMALS[a/]

Organism	Species Affected	Differentiating Characteristics
Toxoplasma	All	Do not stain with grams
"M" organism (Toxoplasma micret.)	Field mouse (Microtus modestus)	Lobulated cysts in brain
Globidium (Besnoitia)	Deer mouse	Large cysts up to 2 mm in diameter
Sarcocystis	All	Basophilic cysts in muscle
Leishmania	All	Kinetoplast
Klossiella	Rat, mouse (guinea pig?)	0.5 to 18 μ All developmental stages present PAS negative H & E positive
Cryptococcus	All	Mucinous capsule 10 μ Organism 5 to 20 μ
Histoplasma	All	Reticuloendothelial response by host PAS positive

a. From Frenkel.[17]

III. DISCUSSION

Nosematosis is a disease of laboratory animals that must be recognized and eventually controlled in order to assure animals of high quality for use in experimentation. Since the disease is clinically silent, recognition is usually accomplished by necropsy. Intraperitoneal inoculation of infected material into mice produces ascites; the organism can be recovered from this fluid. In vitro culture of the organism has not been successful.[16] The combination of lesions in the brain, heart, kidney, liver, and aorta, although not pathognomonic, is highly suggestive of infection by N. cuniculi. The demonstration of the characteristic gram-positive organisms in brain lesions and renal tubular epithelium further supports the diagnosis.

Lainson et al.[12] proposed the pathogenesis of this disease on the basis of electron microscopic studies. They believe that mononuclear and mesothelial cells are infected by the sporoplasm that is extruded from the spore by a long polar filament. They hypothesized that extrusion occurs in nature when the animal swallows the spore and the polar filament "shoots" the sporoplasm into the gut epithelium. Development by binary fission to mature spores occurs in this tissue and the spores are disseminated by vascular routes to other tissues of the body. Lesions caused by the parasite are due to the host response against the free spores. This raises a question as to the source of infective spores. Urine of animals parasitized by N. cuniculi is known to be infective by parenteral inoculation,[13] but the disease has never been satisfactorily produced by oral administration of infected urine. No experiment has been performed that clearly demonstrates the mode of natural transmission of the disease.

Further work is necessary to determine the mode of natural transmission and control of N. cuniculi in laboratory animals and the requirements for in vitro culture.

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13. ABSTRACT

A review of the literature and personal observations on nosematosis of laboratory rodents are presented. The disease, caused by the protozoan Nosema cuniculi, is a spontaneous enzootic entity of laboratory rodents. The presence of occult infections in laboratory animals has confounded the results of numerous experiments. The importance of diagnosing nosematosis in laboratory animals, therefore, cannot be overemphasized. Clinical signs are usually absent, and infection can be recognized only by necropsy or by recovery of the organism following intraperitoneal inoculation of infected material into mice. Gross and histopathological lesions are described for various laboratory animals. The mode of natural transmission of the disease has not been determined. There is a need for further research on transmission and control of nosematosis as well as in vitro culture of the organism.

14. Key Words

Nosematosis
Pathology
Laboratory animals
Cross transmission
Cross infection
Nosema cuniculi
Encephalitozoon cuniculi

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